apparatus (IVIS®). The mouse metatarsal angiogenesis assay (4) was performed to test angiogenic properties of UC-MSC conditioned media (CM) (5) *in vitro*. To provide a screening of cytokines in the CM of UC-MSC, we used a Human Cytokine Array Assay (R&D system).
Results: Histological analysis showed that, in an *in vivo* ectopic model, UC-MSC, contrary to BM-MSC, did not induce bone formation although numerous blood vessels were noted. The UC-MSC angiogenic properties were confirmed by the metatarsal angiogenesis *in vitro* assay. The same angiogenic activity was not observed for BM-MSC. A screening of cytokines secreted by UC-MSC indicated their pro-inflammatory role in the microenviroment. Interestingly, live-cell tracking *in vivo* showed that UC-MSC remained visible for a maximum of 3 weeks after implantation into the scaffold in respect to BM-MSC which remained visible for more than 7 weeks.
Discussion and conclusions: UC-MSC behaved differently than BM-MSC with regard to angiogenic and osteogenic properties. Bone tissue was not formed inside the scaffold seeded with UC-MSC possibly due to a shorter time presence of the cells in the scaffold. Instead, UC-MSC displayed striking angiogenic features making them valuable for therapeutic applications to stimulate regeneration in the case of chronic degenerative processes.
Acknowledgments: Raffaele Spanò, Eugenio Patrone, Barbara Canciani, Elisa Carra.
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OP191
A self-organising biomimetic collagen/nano-hydroxyapatite/GAG scaffold for spinal fusion

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Introduction: Degenerative conditions of the spine are a worldwide problem, leading to chronic and often severe back pain. Spinal fusion is an effective treatment but complications include pseudoarthrosis and donor site pain in cases where an autograft is used, therefore driving the search for alternative treatments[1]. We are recapitulating the extracellular matrix of normal bone using an osteoconductive scaffold together with osteoinductive agents, in an attempt to develop a novel targeted therapy for use in degenerative vertebral conditions.
Materials and methods: A biomimetic strategy was employed to fabricate a collagen/hydroxyapatite/glycosaminoglycan (GAG) scaffold. This was characterised using scanning electron microscopy (SEM), atomic force microscopy (AFM) dynamic scanning calorimetry (DSC), micro-CT, multiphoton spectroscopy and mechanical testing. Scaffolds were seeded with human mesenchymal stem cells (hMSCs). Proliferation assays and quantitative gene expression (using RT-PCR) were performed to study stem cell growth and osteoblastic differentiation (levels of BMP-2, RUNX2, osteocalcin). Student's t-test was used to determined statistical significance ($p < 0.02$).
Results: A self-organising collagen nanohydroxyapatite GAG scaffold was fabricated and micro-CT and SEM confirmed a heterogeneous, porous structure with a mean pore diameter of 150–200 μm (Figure 1a,b). Viable hMSCs were visualised within and throughout the scaffold (Figure 1c). The denaturation temperature of the scaffold was 52°C (Figure 2), therefore above that of body temperature and supporting the potential clinical utility of this scaffold.

RT-PCR showed a significant increase in expression of markers of osteogenesis (BMP-2, RUNX2, osteocalcin) in hMSCs grown in the scaffold compared to those grown on culture plates.

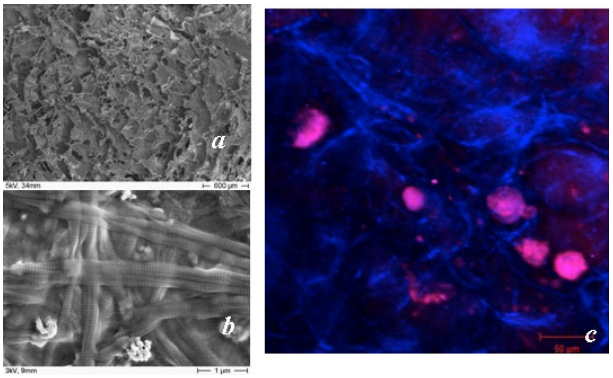


Figure 1 SEM images showing (*a*) pore structure of scaffold and (*b*) fibrillar banding pattern of collagen with homogenous distribution of chondroitin sulphate and nanoHA. (*c*) 2-photon microscopy image of live hMSCs (pink) throughout collagen scaffold (blue).

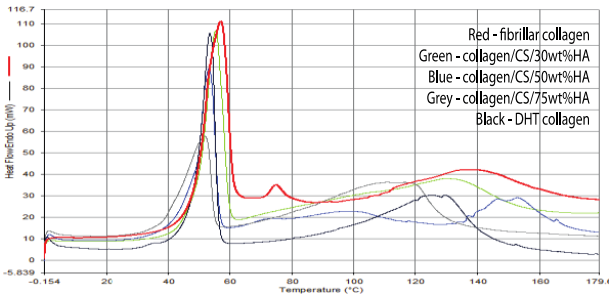


Figure 2 DSC thermogram of fibrillar collagen, collagen/CS/30–75 wt %HA and DHT-crosslinked collagen scaffolds.

Discussion and conclusions: We have successfully synthesised a novel collagen-based scaffold using a biomimetic strategy. This is biocompatible and promotes bone formation. Further studies, including combination with rhBMP-2 and *in vivo* work, are required.
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Disclosures: The authors have nothing to disclose.
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OP192
Marine inspired biomaterials: from sea up to tissue regeneration approaches

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Introduction: Nature has been since ever the inspiration driving mankind for the development of amazing systems, materials, constructions, devices, etc. Marine environment is not exception and the technological developments allowing the access to deeper locations and organisms are opening even more that gate to a new dimension of knowledge. One of the fields where marine organisms are acting stimulating the

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imagination of mankind is health, fostering the development of innovative pharmaceuticals and cosmetics, as well as new biomedical systems [1]. In this presentation, the authors will address the efforts that are being made by 3B's Research Group on the development of marine inspired biomaterials towards tissue regeneration.

Materials and methods: Polymer extraction: Collagen has been extracted from several fish skins by acetic acid extraction, complemented by enzymatic treatment by using pepsin. Polysaccharides have been extracted from different marine sources, namely from algae (sulfated polysaccharides, typically using hot water and precipitation with organic solvents) and from squid pens (chitin, further converted into chitosan, by NaHO treatments).

Development of biomaterials: Porous structures have been developed following different processing techniques, from freeze-drying (chitosan and collagen) to particle agglomeration (chitosan and composites with nanohydroxiapatite) and rapid prototyping (carrageenans). Their morphological properties were assessed by SEM and micro-computed tomography (μCT); mechanical properties evaluated by compression tests; biological performance has been tested with chondrocyte-like cell line ATDC5 (chitosan and collagen scaffolds) or adipose derived stem cells, ASCs (chitosan scaffolds). Additionally, natural collagenous porous structures were obtained by decellularization of marine sponges, together with extraction of toxic compounds.

Results: Several marine origin polymers were used to produce porous structures envisaged as scaffolds for tissue engineering approaches. Squid chitosan scaffolds were produced by freeze-drying, in which ATDC5 cells adhered and proliferated. Squid chitosan scaffolds were produced also by particle aggregation, including as composites with nanohydroxiapatite, in which ASCs were successfully cultured up to 7 days. Carrageenan structures were produced by rapid prototyping, revealing to be non-cytotoxic to rat lung fibroblasts. Marine collagen structures have been produced by freeze-drying and crosslinked with genipin under dense CO_2, obtaining porous structures stable in culture medium, in which ATDC5 cells were successfully cultured.

Additionally, the collagenous structure of marine sponges was also assessed as nature made scaffolds, revealing a non toxic structure able to support culture of osteoblast-like cell line.

Discussion and conclusions: Several porous structures have been produced based on marine origin polymers, and successfully used as 3D support for cell culture. Their use as scaffolds is enhancing the potential of marine resources and inspiration for tissue regeneration approaches.

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OP193
Biocalcite, a multifunctional inorganic polymer: bioseed for the synthesis of calcium phosphate-based bone

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Introduction: Ca-carbonate is the component that builds up the spicules of the calcareous sponges. Recent results revealed that the Ca-carbonate/biocalcite-based spicular skeleton of these animals is formed via an enzymatic mechanism. The enzyme that mediates the Ca-carbonate deposition has been identified as a carbonic anhydrase (CA) and has been cloned from the calcareous sponge species *Sycon raphanus* [1]. Ca-carbonate deposits are also found in vertebrate bones besides the main constituent, Ca-phosphate/hydroxyapatite (HA). Evidence has been presented that during the initial phase of HA synthesis poorly crystalline carbonated apatite is deposited. Recent data indicate that during early bone formation, Ca-carbonate deposits act as potential bioseeds for precipitation of Ca-phosphate mineral onto bone-forming osteoblasts [2–4].

Materials and methods: In this study, we used two permanent cell lines, SaOS-2 cells and RAW 264.7 cells.

Results: By using the human osteogenic SaOS-2 cells it could be shown that after exposure of the cells to Ca-bicarbonate *in vitro*, a significant increase of Ca-deposit formation results. In parallel, the expression of the carbonic anhydrase-II (CA-II) gene becomes upregulated. Finally, it is shown that ortho-phosphate and hydrolysis products of polyphosphate inhibit CA-II activity, suggesting a feedback regulatory system between the CA-driven Ca-carbonate deposition and a subsequent inactivation of this process by ortho-phosphate.

Discussion and conclusions: The data presented here support the view that the Caphosphate/hydroxyapatite deposition reactions in bone tissue are preceded by Ca-carbonate precipitation, a process that is driven by an increased CA activity. The proposed hypothesis, the enzymatic synthesis of Ca-carbonate via CA, leaves room for a future detailed localization of the deposits formed by poorly crystalline Cacarbonate or by carbonated apatite in the vicinity of the plasma membrane. This discovery that Ca-carbonate crystals act as bioseeds in human bone formation may allow the development of novel biomimetic scaffolds for bone tissue engineering. Na-alginate hydrogels, enriched with bicarbonate and biosilica, have recently been demonstrated as a suitable matrix to embed bone forming cells for rapid prototyping bioprinting/3D cell printing applications [5,6].

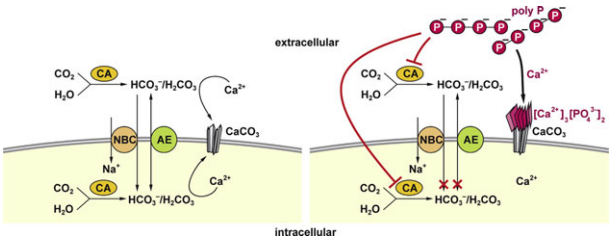


Figure 1 Schematic outline of the sequential deposition of Ca-carbonate and Ca-phosphate.

Disclosures: Authors have nothing to disclose.

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OP194
Ratio of synthetic to natural components in GelrinC®, a hydrogel for cartilage repair, is optimal for chondrogenic differentiation of bone marrow stem cells

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Introduction: GelrinC is a biosynthetic hydrogel composed of polyethylene-glycol-di-acrylate (PEG-DA) and denatured fibrinogen and is used in conjunction with microfracture for the treatment of focal cartilage lesions. Unlike other products, GelrinC acts as a resorbable filler that restricts incoming mesenchymal stem cells (MSC) to its eroding

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